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Seroprevalence of Borrelia burgdorferi sensu lato infection in cattle in an area of Switzerland with previously reported clinical cases

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Seroprävalenz von Borrelia burgdorferi sensu lato Infektion bei Kühen in einem Gebiet der Schweiz in dem klinische Fälle aufgetreten sind

Deutsche Zusammenfassung

Die beiligende Arbeit hat zum Ziel die Seroprävalenz einer Infektion mit Borrelia burgdorferi sensu lato Infektion bei Kühen in einem Gebiet in der Schweiz, in dem klinische Fälle beschrieben worden sind, zu untersuchen. Es wurden Proben von 396 Kühen aus 98 verschiedenen Betrieben gesammelt. Die benötigte Anzahl Proben wurde anhand der Daten des Bundesamtes für Statistik (Repräsentative Viehzählung, 1996) für dieses Gebiet berechnet. Zur serologischen Auswertung wurden ELISA Tests entwickelt, bei denen als Antigen drei schweizerischen Borrelia burgdorferi sensu lato Stämme verwendet wurden und zwar B. garinii (VS 102), B. afzelii (VS 461) und B. burgdorferi sensu stricto (VS 219). Zur Bestimmung der Cut-off Werte zwischen seropositiven und seronegativen Proben zu bestimmen wurden ROC („receiver operating characteristic“) Kurven verwendet. Wir wollten falsch positive möglichst vermeiden und wählten deshalbe einen Cut-off der eine hohe diagnostische Spezifität gewährleistet. Bei der Anwendung der gewählten Cut-offs lag die diagnostische Spezifität und Sensitivität bei 96% und 41% (B.garinii), 98% und 56% (B.afzelii) und 97% und 50% (B.burgdorferi sensu stricto). Die Seroprävalenzen wurden bei B.garinii auf 16.2% (95% Vertrauensintervall: 12.2-20.3%), bei B.afzelii auf 14.4% (10.5-18.4%) und bei B.burgdorferi sensu stricto auf 23.7% (19.1-28.3) geschätzt. Wenn diese Werte in die wahren Prävalenzen umgerechnet wurden, ergaben sich für B.garinii 33%, für B.afzelii 23% und für B.burgdorferi sensu stricto 44%. Lyme borreliosis wird bei Kühen relativ selten diagnostiziert. In Anbetracht der hohen Seroprävalenz ist es möglich, dass die Erkrankung unterschätzt wird.
Die Arbeit liegt als Englischsprachiges Manuskript vor, da sie als Publikation in einer Fachzeitschrift eingereicht werden soll.

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Seroprevalence of *Borrelia burgdorferi* sensu lato infection in cattle in an area of Switzerland with previously reported clinical cases

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Abstract

The seroprevalence of *Borrelia burgdorferi* sensu lato infection of cattle was studied in an area of Switzerland were clinical cases of Lyme borreliosis have been reported. To this end, ELISA tests were established using antigens prepared from *B. afzelii*, *B. garinii* and *B. burgdorferi* sensu stricto, respectively. To determine cut-off values defining seropositive and negative samples, ROC curves were used. When optimal cut-offs were determined, the diagnostic specificity and sensitivity were 96% and 41% (*B. garinii*), 98% and 56% (*B. afzelii*) and 97% and 50% (*B. burgdorferi* sensu stricto). The seroprevalences were estimated to be 16.2% (95% Confidence interval: 12.2-20.3%) for *B. garinii*,

14.4% (10.5-18.4%) for *B. afzelii* and 23.7% (19.1-28.3%) for *B. burgdorferi sensu stricto*, respectively. These were translated into true prevalences of 33% for *B. garinii*, 23% for *B. afzelii* and 44% for *B. burgdorferi sensu stricto*. Lyme borreliosis as a disease is only rarely diagnosed in cattle. In view of its relatively high seroprevalence Lyme borreliosis may be underestimated.

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Introduction

Lyme borreliosis (LB) or Lyme disease is a systemic infection caused by the spirochete *B. burgdorferi* sensu lato, which is transmitted by *Ixodes* ticks. In Europe, the vector is *Ixodes ricinus*. Three to 49% of ticks in Switzerland were found to be infected with *B. burgdorferi* sensu lato, depending on the geographical area (18, 28, 39, 41). Lyme borreliosis represents a global public health problem (2) and is the most frequent vector-borne disease in North America (31, 34) and Eurasia (26, 40). In 1977, numerous cases of rheumatoid-like arthritis were described in children in Lyme, Connecticut (33) ; the condition was subsequently called Lyme disease. In 1981, Dr. Willy Burgdorfer identified the cause as a new species of *Borrelia* (4), which in 1984 was named *B. burgdorferi*. Since its first description several additional species have been described. Today, at least ten different species have been identified which are summarized under the term *B. burgdorferi* sensu lato. Representatives include *B. burgdorferi* sensu stricto (USA, Europe), *B. garinii, B. afzelii, B. valaisiana* and *B. lusitania* (Europe and Asia), *B. japonica, B. tanukii* and *B. turdi* (Japan), *B. andersonii* and *B. bissettii*. (USA, Slovenia) (38). Not all of these species are pathogenic to humans. The species most frequently isolated from human patients with LB are *B. burgdorferi* sensu stricto*, B*. *afzelii* and *B. garinii*. The importance of *B. bissettii*. and *B. valaisiana* is not yet known.

Lyme borreliosis has been reported in a number of domestic animals including dogs (20, 22, 29, 35) cats (24), horses (8, 10, 12), sheep, (11, 13, 15) and cattle (6, 17, 27). Clinical signs include musculoskeletal problems of varying

severity such as lameness, swollen joints and arthritis with or without fever. In cows, weight loss, decreased milk production, erythematous dermatitis and abortion have been described (9, 10, 27, 30). Subclinical Lyme borreliosis is common in domestic animals, and the pathogenesis of the disease is poorly defined.

Diagnosis of Lyme borreliosis is not straightforward and is based on clinical signs, ruling out other diseases, results of serology and response to antibiotic therapy. Although serology can be helpful in confirming a diagnosis, a positive result is primarily indicative of a previous contact with the organism, but not necessarily of an active clinical infection. A definitive diagnosis requires isolation of the agent from infected tissue. However, often the organism can not readily be isolated and tissue culture is demanding and time consuming. Identification of *B. burgdorferi* sensu lato DNA via polymerase chain reaction (PCR) is another diagnostic test, although the significance of DNA identification in the absence of live bacteria is not entirely clear.

Recently *B. burgdorferi* sensu lato infection was diagnosed in two cows originating from an area in the south west of Zurich, Switzerland (19). Clinical signs included erythematous lesions on the skin of the udder, poor general condition, decreased milk production, stiff gait and swollen joints. *B. burgdorferi* sensu lato DNA was detected in synovial fluid in both cows and in the milk from one of the cows.

It was the objective of the present study to determine the seroprevalence of *B. burgdorferi* sensu lato infection in cattle from the same region where these two cases had been diagnosed.

Materials and methods

Farms and cows

This study was conducted under the permit number 147/2000 for animal research obtained from the state of Zurich in compliance with the federal animal welfare laws.

Exposed group

Serological testing was performed on 396 cattle from 98 farms located in a region of Switzerland where *Ixodes ricinus* are common and two cases of Lyme borreliosis were diagnosed previously (19). The required number of serum samples for this region was calculated using data from the Federal Bureau of Statistics based on the representative census of farm animals in 1996. The cow population in the selected area was 5330 animals. The number of samples was calculated to estimate a prevalence of 20% with 5% precision at a 95% confidence level. Therefore, the target number of samples was 300. In each herd, a minimum of 3 animals were sampled to detect at least one positive animal with 95% certainty (assumption: herd size n=20, within-herd prevalence =75%), Samples were collected in the fall of the year 2000. The cattle were of various ages and breeds and were chosen randomly on each farm. The only prerequisite was that the animal had been on the farm for a minimum of one

year. The herd size on each farm varied from seven to 64 cattle (mean 22). A questionnaire was completed for each farm to obtain information on the following items: type of barn (free stall, stanchions), type of pasture (bordering a woodlot, hedges, trees) and occurrence of ticks (yes, no) on cows.

Control group

Serological testing was performed on 157 cattle from 37 farms located in a region of Switzerland where *Ixodes ricinus* had never been identified (alpine regions; >1400 m above sea level; Landschaft Davos, Hinterrhein, Avers). The cattle in this group had never been outside of this region. These herds represented a convenient sample within the region.

Blood Samples

Whole blood was collected from the coccygeal blood vessels and allowed to clot at room temperature. Samples were then centrifuged at 1000*g* for 10 min after which time the serum was removed and stored at -20°C until further analysis.

Positive control serum samples

A mixture of three sera that reacted strongly in preliminary testing was used as positive control samples. They were tested by Western Blot analysis and yielded at least the following specific bands: 31kDa (OspA), 34Da (Osp B) and 39kDa.

Western Blot

Western Blot analysis was carried out as described elsewhere (21) using 5μg protein antigen per lane prepared from three Swiss isolates of *B. burgdorferi* sensu lato.

Isolates of *Borrelia burgdorferi* sensu lato

The isolates used in this study were *B. burgdorferi* sensu stricto (VS 219), *B. afzelii* (VS 461) and *B. garinii* (VS 102) (28), which were cultured in Barbour-Stoenner-Kelly-II-medium (Sigma, Division of Fluka Holding AG, CH – Buchs) with 10% rabbit serum at 37°C and 5% CO_2 (1). The bacteria were washed by centrifugation (15'000 *g* for 15 min at 4° C) three times using phosphate buffered saline (PBS) containing 5mM $MgCl_2$. The bacteria were transferred into 1 to 2 ml of 0.1% Sodium-dodecyl-sulfate (SDS) solution, which was heated to 95°C for 5 min and disrupted in an ultrasonic bath for 15 min.

Determination of protein concentration

The concentration of protein was determined using the Micro BCA® Protein Assay Reagent Kit (Pierce, Rockford, USA).

Enzyme-linked immunosorbent assay

The ELISA plates (ELISA F-plates Immulon® M129A; Microtec Producte AG, Embrach, Switzerland) were coated with 100 ng per well of the respective bacteria in 0.1M Na-Carbonate buffer, pH 9.6, incubated for 3 hours at 37°C and

stored overnight at 4°C. Afterwards, the plates were stored at -20°C until further use.

The plates were washed three times with ELISA wash solution (0.15M NaCl, 0.05% Tween 20) and the serum was diluted 1:100 in dilution buffer (50 mM Tris-HCl, pH 7.4; 0.15M NaCl; 0.1% Tween 20; 1g/l bovine Serumalbumin (BSA) and 1 mM Sodiumethylendiamintetraacetat (Na_2EDTA)) and pipetted into the wells in duplicate. After incubation of the plates 1 hour at 37°C the wells were washed using a squirt bottle and ELISA wash solution and incubated with 100µl of a rabbit anti-bovine IgG preparation conjugated to peroxidase (Nordic Immunological Laboratories, Tilburg, The Netherlands), in a dilution of 1:1000 in dilution buffer. The plates were incubated for 1 hour at 37°C and then washed as described above. One hundred microlitres of freshly prepared substrate solution (50mM citric acid adjusted to pH 4.0 with 1N NaOH, 2mM H_2O_2 and 0.2mM 2,2-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid); Fluka Holding AG) was then added to each well. After 15 min, the absorbance value was measured at 405 nm using an ELISA Plate Reader Dynatech MR 700. Positive control sera were included in three duplicates with each plate. Dilution buffer in three duplicates was used as a conjugate control.

Analysis of results

The concentration of antibody was expressed as relative reactivity. For each plate, the difference between the absorbancy of the sample and that of the conjugate control was expressed as a percentage of the difference between the absorbancy of the positive control and that of the conjugate control:

Relative reactivity (%) =

$(OD_{sample}-OD_{conjugate\ control})/(OD_{positive\ control}-OD_{conjugat\ control}) \times 100$

Determination of the cut-off value

A receiver-operating-characteristic (ROC) analysis was performed to determine the cut-off value of relative reactivity associated with *B. burgdorferi* sensu lato infection (14). Definition of the cut-off value would usually require a group of known seropositive individuals and a group of known seronegative individuals. As there was no reference test available, the following approximation was used: It was assumed that the degree of serologic crossreactivity induced by infectious agents immunologically related but not identical with *B. burgdorferi* sensu lato (e.g. *Leptospira spp*., *Treponema spp*.) would be similar in both groups of cattle, the exposed group and the control group. Thus, the relative frequency of the absorbance classes of the control group was subtracted from that of the exposed group. The remaining frequencies observed in the different absorbance classes of the exposed group were used to calculate the absolute number of animals in the different absorbance classes; this values were utilized as the positive population in the ROC analysis.

The ROC analysis was performed using the software program WinEpiscope 2.0 (free download under http://www.clive.ed.ac.uk/winepiscope/) .

Ticks

In the fall of 2001, a total of 75 *Ixodes ricinus* ticks was collected in the above mentioned region of Switzerland. Collection was done using an umbrella

that was covered with a terry cloth towel and repeatedly pushed through the low underbrush present in the study region. The ticks were stored in individual Eppendorf tubes at –20 °C until they were used for detection of *B. burgdorferi* sensu lato DNA.

PCR

B. burgdorferi sensu lato DNA was amplified by the real-time PCR described elsewhere (18).

Statistics

The prevalences of the different agents were calculated with their 95%-confidence intervals. Individual animal prevalences were adjusted for clustering as described by McDermott et al. (1994)(25). The resulting apparent prevalences were transformed into true prevalences using the following formula:

True prevalence = [apparent prevalence –(1-specificity)]/[1-{(1-Specificity)+(1-sensitivity)}]

In order to determine whether a significant association existed between seropositivity to the three *B. burgdorferi* sensu lato species and factors favoring existence of ticks the results of the questionnaire were evaluated by conducting chi-square test and logistic regression using the computer program StatView 5.0 (SAS Institute Inc.).

To determine whether an isolate specific immunereaction to different *B. burgdorferi* sensu lato strains existed in individual cows, regression plots were

established and correlation coefficients were determined using the least square method using StatView 5.0.

Results

Frequency distribution of absorbance values

All serum samples were tested using the three antigens.

The results are shown in figure 1.

ROC Curves and prevalences

Results of the ROC analysis are displayed in figure 2.

Ideally serological tests for the detection of antibodies specific for different species of *B. burgdorferi* sensu lato should have high specificity and sensitivity. As this is usually not possible a compromise has to be made. For the purpose of our study we determined a cut-off value that yielded a high specificity (>95%) in order to avoid false positive results which in turn gives rise to a low sensitivity.

In order to determine the prevalence of antibodies specific for the three *B. burgdorferi* sensu lato species the ROC curves of figure 2 were utilized. Using a cut-off value of 80% of the absorbancy shown in figure 1 the following diagnostic specificity's and sensitivities were obtained: 96% and 41% (*B. garinii*), 98% and 56% (*B. afzelii*) and 97% and 50 % (*B. burgdorferi* sensu stricto).

Using these conditions the percentage of seropositive results were found to be as shown in table 1.

The true prevalences calculated from the results in table 1 were 33% for *B. garinii*, 23% for *B. afzelii* and 44% for *B. burgdorferi* sensu stricto.

Immunological relationship between the three different *Borrelia burgdorferi* sensu lato strains

Immunological relationship between the three *B. burgdorferi* sensu lato preparations used in this study were evaluated by the calculation of correlation coefficients and establishment of regression plots using the absorbance values obtained in the respective ELISA(Fig. 3). The correlation coefficients for *B.afzelii* and *B.burgdorferi* sensu stricto was found to be 0.695 with a p-value <0.0001, for *B.afzelii* and *B.garinii* 0.814 and a p-value <0.0001 and for *B.burgdorferi* sensu stricto and *B.garinii* 0.744 ($p < 0.0001$), respectively. It becomes evident that for the majority of cases a strong correlation exists between the ELISA results obtained from each of the assays done. With the possible exception of a few outliers (areas a, b, c in Fig. 3a-c) all the samples appear to belong to one population.

Risk factors associated with seropositivity

The parameters determined from the questionnaire were evaluated for significant association with seropositivity. No significant association was found between presence of hedges, trees and edge of forest on the pasture compared with absence of these conditions. Furthermore, no correlation was found between the parameter “ticks seen on cattle” and “ticks seen on cats and dogs at the same farm” (Data not shown).

Age distribution

The age distribution of cattle seropositive for the various *B. burgdorferi* sensu lato strains is shown in table 2. There were no significant differences between the age groups.

Ticks

From the 75 examined ticks 20 were positive in the real-time PCR.

Discussion

B. burgdorferi sensu lato infections are infrequently diagnosed in cattle and their significance is not clear. Although clinical signs are usually not specific for Lyme borreliosis and therefore are not diagnosed as a disease caused by *B. burgdorferi* sensu lato infection, there are a few well described cases of Lyme borreliosis in cattle (7, 27, 19). Subclinical infection has been reported in cattle and other domestic animals (10). In cattle, *B. burgdorferi* sensu lato has been isolated from blood, synovia, milk, colostrum, urine and other tissues via culture and PCR (9, 17, 19). Shedding of *B. burgdorferi* sensu lato in urine has been described in cows (6), white-footed mice (*Peromyscus leucopus*) (3) and horses (23). Infection has been reproduced experimentally in *Peromyscus maniculatus* after oral inoculation (5). Burgess postulated that infection could be transmitted horizontally among cows by infected urine contacting mucous membranes (9). Intrauterine infection of a fetus of a naturally infected cow has been reported and represents another route of transmission (17). Transmission of *B. burgdorferi* sensu lato via infected milk is not only a potential route of infection for calves but in theory also for humans.

This study has shown that in a region of Switzerland, in which the vector of *B. burgdorferi* sensu lato (*Ixodes ricinus* ticks) occurs, the seroprevalence of this infection in cattle was estimated to be 33%, 23% and 44% for *B. garinii, B. afzelii* and *B. burgdorferi* sensu stricto, respectively. The cut-off to distinguish between antibodies specific or not specific for *B. burgdorferi* sensu lato was determined using the ROC procedure which has been introduced to the use for

B. burgdorferi sensu lato epidemiology only recently (32). This procedure allows the objective determination of a cut-off with the desired degree of diagnostic specificity and the correspondent sensitivity. There are cross-reacting antigenic determinants among various *B. burgdorferi* subspecies, but also between *B. burgdorferi* sensu lato and other bacteria (e.g., spirochetes). It has been reported in experimental *B. burgdorferi* sensu lato infection in cattle that the serologic reaction was more or less specific for the *B. burgdorferi* species used to infect individual animals (37). In order to make sure that we would not miss by chance seropositive animals we used in the present study antigens prepared from the three species of *B. burgdorferi* sensu lato known to occur in Switzerland. Based on the positive correlation of antibodies to the different species of *B. burgdorferi* (Fig. 3) we concluded – in contrast to the study conducted in Sweden (36)– species specific antibodies do not occur in natural *B. burgdorferi* sensu lato infection in cattle or that our test would not detect these antibodies. There were a few animals that showed a marked dichotomy in the results to the different strains used for the ELISA (Fig. 3; outliers a, b, c). Although we did not determine by what agent seroconversion had been induced in these animals, it may be speculated that in these cases the infecting agent was *B. afzelii* (outliers a and c) and *B. burgdorferi* sensu stricto (outlier b). Among the seropositive cattle antibodies to *B. burgdorferi* sensu stricto were most prevalent. It is known that expression of OspC and OspA differ in their extend in different strains of *B. burgdorferi* sensu lato. For instance the strain VS 102 of *B. garinii* used in the present study is known to express large amounts of OspC but not of OspA.

Therefore this strain is used for serodiagnosis of Lyme borreliosis in humans (O.Péter, personal communication). We did not study whether or not the different Osps of the three *B. burgdorferi* sensu lato species are recognized preferentially by the bovine immune system. Preferential recognition of different Osps could be an explanation for the differences in absorbance values observed in our three ELISA systems.

The above-stated estimates of the seroprevalence are not exact values but approximations of the true prevalence. The diagnostic sensitivity and specificity of the tests had to be derived using a relatively small control group from an area where the vector was never observed. More precise values of the test characteristics could have been derived if sera from experimentally-infected animals were available. We also can not fully exclude that the difference in antibody levels between the exposed and the control population can be explained entirely by antibodies to *B. burgdorferi* sensu lato. It could also be that in the population of exposed animals other cross-reacting agents were present at a higher prevalence that may have given rise to an increased concentration of antibodies. In that latter case the prevalence found here would have been overestimated.

That the relatively high seropositivity to the three different strains of *B. burgdorferi* sensu lato is indeed caused by *B. burgdorferi* sensu lato is supported by the fact that 20 out of 75 ticks found in the very same area tested positive by PCR for *B. burgdorferi* sensu lato DNA. Further proof that our ELISA detects antibodies directed against *B. burgdorferi* sensu lato is provided by the

observation that in a follow-up study 5 out of 98 cows that were tested monthly over one grazing period tested positive for *B. burgdorferi* sensu lato DNA in milk samples (Data not shown; work in progress).

The seroprevalence found in the present study was directly comparable to data provided in two studies in Japan (16, 36). The observation that no significant association between seropositivity and the parameter “hedges”, “trees” and “edge of forest” was found may be explained by the fact that seropositive animals may have been infected during previous seasons on different pastures. The infestation of cows with ticks was rarely reported by the owners. Even on the farm on which Lyme borreliosis was reported in a cow three years previously (19), ticks had never been observed by the owner on any of his cattle. This fact could be explained by the following: (i) It is possible that ticks were missed because generally, cattle are not examined for ticks as thoroughly as other domestic animals. In addition some of the farmers reported that calves and heifers had been found to be more frequently infested with ticks than cows. (ii) For transmission of infection by *B. burgdorferi* sensu lato routes other than tick bites should be considered such as the urine-oral route as described (9).

The seropositive cattle in this study did not have clinical signs of Lyme borreliosis such as lameness or swollen joints. As we did not test for presence of the infectious agent it is unknown whether the cattle had a subclinical infection or whether seropositivity was the consequence of a transient infection. However, there is no doubt that *B. burgdorferi* infection has some importance in the

population studied in this report. As demonstrated, *B. burgdorferi* sensu lato infection occasionally can lead to severe clinical signs (19).

Although at present we do not have evidence of any risk of transmission of *B. burgdorferi* sensu lato infection by milk, in view of the prevalence found here and the clinical observations made in cattle originating from the area studied, one of the questions that now should be addressed, is the safety aspect of this infectio n in connection with cattle as a source of milk and meat.

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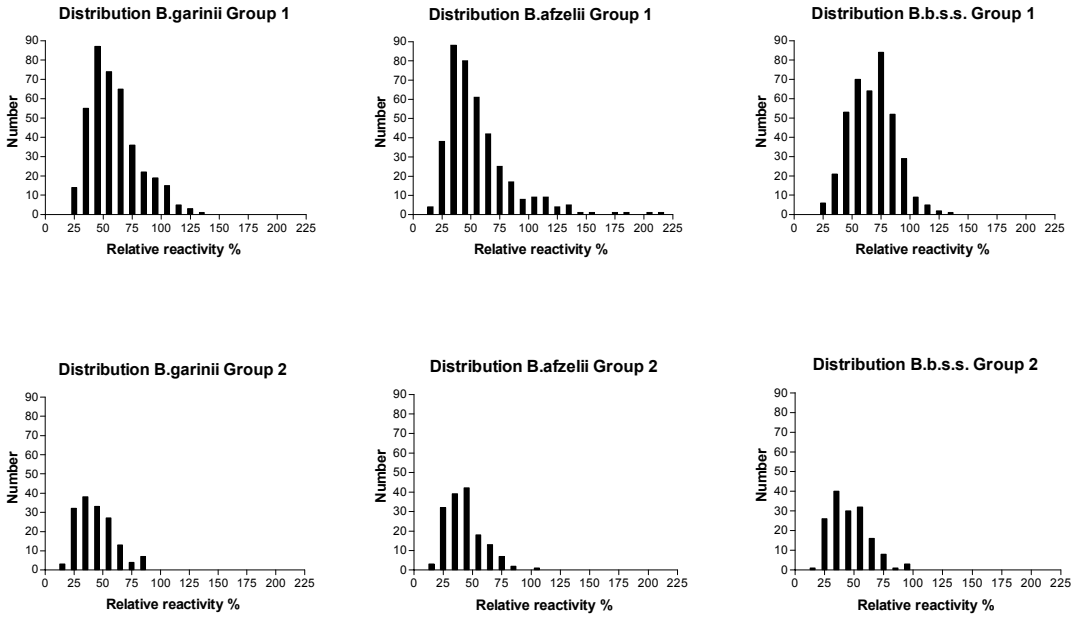
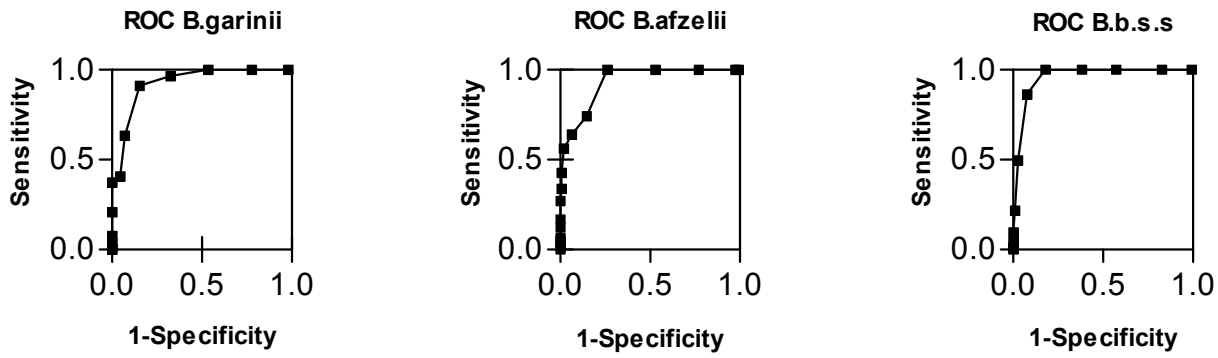

Distribution B.garinii Group 1
Distribution B.afzelii Group 1
Distribution B.b.s.s. Group 1
Distribution B.garinii Group 2
Distribution B.afzelii Group 2
Distribution B.b.s.s. Group 2
Number
Relative reactivity %
0 10 20 30 40 50 60 70 80 90
0 25 50 75 100 125 150 175 200 225

Figure 1


ROC B.garinii
Sensitivity
1.0
0.5
0.0
0.0
0.5
1.0
1-Specificity
ROC B.afzelii
Sensitivity
1.0
0.5
0.0
0.0
0.5
1.0
1-Specificity
ROC B.b.s.s
Sensitivity
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0.5
0.0
0.0
0.5
1.0
1-Specificity

Figure 2

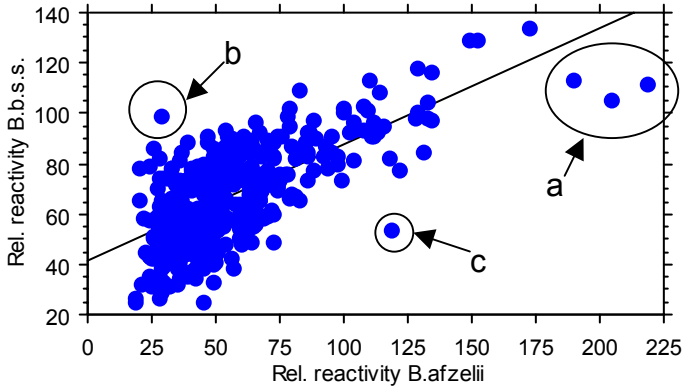

Fig. 3a

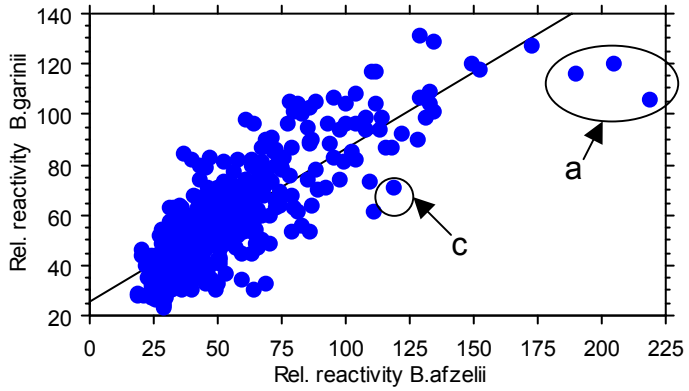

Fig. 3b

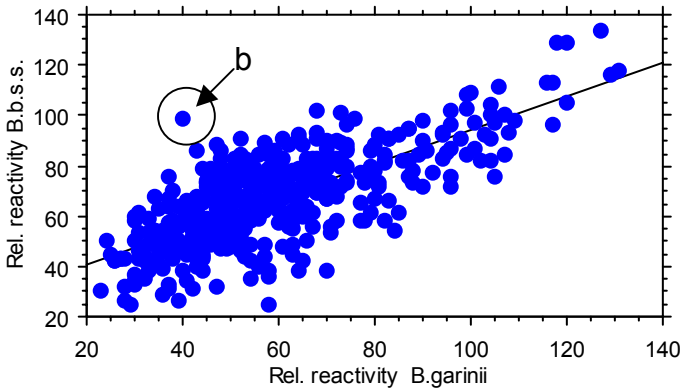

Fig. 3c

Table 1: Compilation of apparent prevalences of antibodies to the three species of *Borrelia burgdorferi* sensu lato[1]

	B.b.s.s		***B.garinii***		***B. afzelii***	
No. of seropositive animals [n=396]	94	*23.7%*	64	*16.2%*	57	*14.4%*
No. of farms with at least one seropositive animal [n=98] (95% C.I.)	62	*63.3% (53.4-72.6%)*	46	*46.9% (36.1-55.9%)*	41	*41.8% (31.2-50.8%)*
No. of farms with No. of seropositive animals/farm						
0/4	37	*37.7%*	52	*53.1%*	57	*58.2%*
1/4	37	*37.7%*	33	*33.7%*	30	*30.6%*
2/4	15	*15.4%*	8	*8.1%*	6	*6.1%*
3/4	9	*9.2%*	5	*5.1%*	5	*5.1%*

[1] Cut-offs were set as described in the text

Table 2: Age distribution

	Number of seropositive samples against					
Age groups (years)	***B.b.s.s.***		***B. garinii***		***B. afzelii***	
2-4	36/160	22.5%	20/160	12.5%	23/160	14.4%
5-7	42/159	26.4%	30/159	18.9%	24/159	15.1%
8-10	13/65	20%	10/65	15.4%	7/65	10.8%
11-14	3/12	25%	4/12	33.4%	3/12	25%

Legends:

Figure 1: Frequency distribution of absorbance values

The frequency distribution of absorbance values obtained in 3 ELISAs using antigen of *B. garinii, B. afzelii* and *B. burgdorferi* sensu stricto are shown with the serum samples of cattle of group 1 exposed to ticks and of group 2 never exposed to ticks. It is evident that with all three antigens a sizeable portion of the serum samples of animals of group 1 yielded higher absorbance values than those of group 2.

Figure 2: ROC Curves

Absorbance values of the ELISA's done with three antigens and serum samples of cattle of group 1 and 2. The ROC curves were calculated using absorbance values. They allow the objective determination of a cut-off which defines a high diagnostic specificity (>95%). Using this cut-off the diagnostic sensitivity was 41%, 56% and 50% for *B. garinii*, *B. afzelii* and *B. burgdorferi* sensu stricto respectively.

Figure 3: Immunological relationship between different strains of *B. burgdorferi* sensu lato in naturally infected cattle

OD Results obtained with individual sera using different antigen preparations.

Fig. 3a: Relationship between *B. burgdorferi* sensu stricto and *B.afzelii*

Fig. 3b: Relationship between *B. garinii* and *B. afzelii*

Fig 3c: Relationship between *B.burgdorferi* sensu stricto and *B. garinii*

It becomes evident that there exists a general correlation between the ELISA results obtained with different antigen preparations.

a, b, c: outliers

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Lebenslauf

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